File: 12067-0003

IN THE UNITED STATES PATENT AND TRADEMARKS OFFICE

In The Matter Of Patent Application:

Applicant	:	Michael J. ROCHON
Assignee	:	Virox Technologies Inc.
Serial No.	:	10/067,809
Filing Date	:	February 8, 2002
Title	:	HYDROGEN PEROXIDE DISINFECTANT WITH INCREASED ACTIVITY
Examiner	:	John Pak
Art Unit	:	1616
Paper No.	:	

To: Assistant Commissioner for Patents
The U.S. Department of Commerce,
PATENT OFFICE
Washington, D.C., U.S.A., 20231

AFFIDAVIT OF NAVID OMIDBAKHSH

I, Navid Omidbakhsh, of the City of Mississauga, in the Regional Municipality of Peel, MAKE OATH AND SAY:

1. I am the current Director of Research and Development at Virox Technologies Inc., the assignee of the present invention. I have a Bachelor of Science degree in Chemical Engineering and have worked in the chemical industry for more than 7 years. I have reviewed U.S. application serial number 10/067,809 filed on February 8, 2002, the prior art of record in this application, the Office actions dated December 31, 2002 and

July 9, 2003, the response filed on March 31, 2003 and the response being filed concurrently herewith. As such, I have personal knowledge of the following matters to which I depose.

2. I have conducted experiments to study the contribution of various anionic surfactants towards the efficacy of the present solution. Solutions X1-A to XI-H (summarized in Table XI-1 below) were tested and the results are summarized in Table XI-2 below.

Table XI-1

Lot#	XI-A	XI-B	XI-C	XI-D	XI-E	XI-F	XI-G	XI-H
Raw Material	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w	% w/w
Deionized water	98.960	98.750	98.600	98.960	98.590	98.540	98.690	98.690
Phosphoric acid (75%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Briquest ADPA-60AW (60%)	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
C6-Dowfax Hydrotrope (45%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Alfonic L610-3.5 (100%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Biosoft S-100 (98%)	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dowfax C10L C10 (45%)	0.00	0.39	0.00	0.00	0.00	0.00	0.00	0.00
Petro ULF (ANS) (95%)	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.00
Stepanol WAC (SLS) (29%)	0.00	0.00	0.00	0.00	0.55	0.00	0.00	0.00
Hostapur SAS-30 (30% SAS)	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.00
Stepan mild SL3 (SLSS) (32%)	0	0	0.54	0	0	0	0	0
Bioterge PAS 8 (SOS) (38%)	0.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00
Alpha-Step MC-48 (37% SMSE/SFA)	0	0	0	0	0	0	0	0.42

NOTE

Phosphorus-based acids

- **H_3PO_4** = phosphoric acid
- **STPP** = sodium tripolyphosphate
- **BRIQUEST ADPA-60AW (HEDP)** = 1-hydroxyethylidene-1,1,-diphosphonic acid
- **Briquest 301-50A (ATMP)** = amino tri(methylene phosphonic acid)

Anionic surfactants

- **Biosoft S-100 (DDBSA)** = dodecyl benzene sulfonic acid
- **Dowfax C10L C10** = C10 alkylated sulfonated diphenyl oxide disodium salt
- **Petro ULF (ANS)** = sodium alkyl naphthalene sulfonate
- **Bioterge PAS-8 (SOS)** = sodium octyl sulfonate
- **Hostapur SAS-30** = sodium C14 - C17 sec-alkylsulfonate
- **Stepanol WAC (SLS)** = sodium lauryl sulfate
- **Standapol LF (SOS)** = sodium octyl sulfate
- **Stepan Mild SL3 (SLSS)** = disodium laureth sulfosuccinate
- **DOWFAX hydrotrope** = C6 alkylated sulfonated diphenyl oxide disodium salt
- **Alpha-Step MC-48 (SMSE/SFA)** = solution containing SMSE and SFA (relative ratio of components not given by manufacturer)
  - SMSE = sodium methyl 2-sulfo C_{12}-C_{16} ester
  - SFA = disodium 2-sulfo C_{12}-C_{18} fatty acid salt

Non-ionic surfactants (emulsifiers)

- **Alfonic L610-3.5** = C6 - C10 alkyl, 3.5 moles of ethylene oxide (EO) alcohol ethoxylate (AE)
- **TRITON X-405 (OPE)** = octyl phenol ethoxylate

Anionic surfactants (hydrotropes)

- **C6 DOWFAX hydrotrope** = C6 alkylated sulfonated diphenyl oxide disodium salt

Table XI-2: The activity of various solutions against *Staphylococcus aureus* (Quantitative Carrier Test 1)

Sample	# of Carriers	Dilution	Contact Temp.	Contact Time	CFU/ control Carrier	CFU/ test Carrier	Log_{10} Red'n
XI-A	4	Full strength	RT	3 min	1.02×10^7	5.3×10^5	1.29
XI-B	3	Full strength	RT	3 min	9.20×10^6	6.10×10^4	2.18
XI-C	3	Full strength	RT	3 min	9.20×10^6	7.17×10^5	1.12
XI-D	3	Full strength	RT	3 min	9.20×10^6	5.03×10^5	1.27
XI-E	3	Full strength	RT	3 min	9.20×10^6	4.00×10^5	1.43
XI-F	4	Full strength	RT	3 min	6.76×10^6	6.15×10^5	1.04
XI-G	4	Full strength	RT	3 min	6.76×10^6	1.52×10^5	1.64
XI-H	4	Full strength	RT	3 min	8.13×10^6	9.30×10^4	1.94

3. The results contained in Table XI-2 above can be compared with the results contained in Tables A and C of the affidavit of Jose Ramirez sworn March 31, 2003 (the "Ramirez Affidavit"). The results for Solutions A3 (Table A of the Ramirez Affidavit), XI-A to XI-H (above) and C1 to C9 (Table C of the Ramirez Affidavit) show that the hydrogen peroxide and selected anionic surfactant act synergistically as against *Staphylococcus aureus*.

4. Additional solutions B4, B5, D4 and D5 summarized in Table XII-1a and XII-1b were tested and the results are summarized in Table XII-2 below.

Table XII-1a – Solutions in accordance with the present invention

	B4	B5
Ingredient	%w/w	%w/w
Briquest 543-45AS	0.29	-
Briquest 221-50 A	-	0.29

C6 Dowfax Hydrotrope (45%)	0.08	0.08
Alfonic L610-3.5 (100%)	0.05	0.05
Hydrogen Peroxide (50%)	0.55	0.55
Biosoft S-100 (DDBSA) (98%)	0.18	0.18
Water	To 100	To 100
pH	About 2	About 2

NOTE

Briquest 543-45AS: Diethylenetriaminopenta(methylene phosphonic acid)

Briquest 221-50 A: 2-hydroxyethylimino bis(methylene phosphonic acid)

Table XII-1b – Solutions NOT in accordance with the present invention

	Formulation	
	D4	D5
Ingredient	%w/w	%w/w
Phosphoric acid (75%)	0.11	0.11
Briquest ADPA-60-AW (60%)	0.29	0.29
C6 Dowfax Hydrotrope (45%)	0.08	0.08
Alfonic L610-3.5 (100%)	0.05	0.05
Hydrogen Peroxide (50%)	-	0.55
Biosoft S-100 (DDBSA) (98%)	0.18	-
NaOH (50%)	To pH=6	To pH=6
Water	To 100	To 100

Table XII-2: The activity of Virox products against *S. aureaus* (Carrier test method)

Lot Number	Dilution	# Of Carriers	Contact Time	CFU/ control Carrier	CFU/ test Carrier	Log_{10} Red'n
B4	Full strength	4	3 min	1.03×10^6	0	6.01
B5	Full strength	4	3 min	1.03×10^6	0	6.01
D4	Full strength	4	3 min	1.02×10^7	8×10^3	3.11
D5	Full strength	4	3 min	1.02×10^7	7.75×10^6	0.912

5. The results for B4 and B5 demonstrate that other phosphorus-based acids will achieve the same synergy as achieved by solutions B1, B2 and B3 of the Ramirez Affidavit.

6. The results for D4 and D5 demonstrate that the synergistic results achieved by solutions D1, D2, and D3 of the Ramirez Affidavit cannot be achieved without both the hydrogen peroxide and anionic surfactant being present.

7. This affidavit is made for the purpose of responding to the office action dated July 9, 2003 and for no improper purpose.

Sworn before me in the City of Toronto,)
Province of Ontario,) SS:
this 9th day of November, 2003.)

Navid OMIDBAKHSH

Dolly Kao, Notary Public

Table 9–7. *Effect of pH on Antibacterial Activity of Peracetic Acid*

pH	Pseudomonas aeruginosa ATCC 15442	Klebsiella pneumoniae ATCC 4352	Streptococcus faecalis ATCC 10541	Staphylococcus aureus ATCC 6538
Bacteriostatic (minimum inhibitory concentration in ppm)				
5.0	21	21	21	no growth
6.5	21	21	21	21
8.0	42	42	42	21
Bactericidal (lethality in less than 1 minute at concentration in ppm)				
5.0	42	42	42	42
6.5	42	42	42	84
8.0	42	42	84	84

Baldry, M.C.G. 1983. The bactericidal, fungicidal, and sporicidal properties of hydrogen peroxide and peracetic acid. J. Appl. Bacteriol., 54, 417–423.

cohols has been studied. Werner and Wewalka (1973) observed the sporicidal effect of 0.05% PAA in 70% ethanol and of 0.025% PAA in 60% isopropanol, even in the presence of large amounts of garden soil. Using the resistant spores, *B. sublitis* SA22, Leaper (1984b) reported a synergistic effect of PAA with alcohols. With 0.08% PAA alone the D-value was 47.2 minutes, whereas with the combination of 0.08% PAA and 9.9% methanol, ethanol, or propanol-1, the D-values were 17.3, 4.7, and 1.6 minutes, respectively. Synergistic effects were observed with 0.04% and 0.08% PAA when up to 20% ethanol was used. Higher concentrations of ethanol or peracetic acid did not improve the sporicidal action. The simultaneous use of PAA and ethanol in aseptic packaging was suggested.

Table 9–8. *Effect of pH on the Cidal Activity of Peracetic Acid on Yeasts (ppm for complete kill in less than 5 minutes) at 25° and 4°C*

	Saccharomyces cerevisiae NCYC 762		Saccharomyces cerevisiae NCYC 1026	
pH	25°C	4°C	25°C	4°C
5.0	83	830	42	415
6.5	83	415	42	415
8.0	415	830	83	415

Baldry, M.C.G. 1983. The bactericidal, fungicidal, and sporicidal properties of hydrogen peroxide and peracetic acid. J. Appl. Bacteriol., 54, 417–423.

Table 9–9. *Effect of pH on Antimicrobial Activity of Peracetic Acid (minimum concentration to kill in ppm)*

	pH		
Organism	5	7	9
E. coli	20–25	10–15	100–150
Streptococcus faecalis	10–15	75–100	500–1000
Phage MS2	11–15	30–53	225–300
Phage ϕ, 174	15–23	53–75	525–750

Baldry, M.G.C., French, M.S., and Slater, D. The activity of peracetic acid on sewage indicator bacteria and viruses. Water Sci. Tech.

Table 9–10. *Comparison of Peracetic Acid with Other Disinfectants Against Food-poisoning Bacteria: Effect of Temperature and Concentration in ppm to Obtain Lethality in 5 Minutes*

Organism	Peracetic acid	Active Chlorine	Benzalkonium Cl
20°C			
Listeria monocytogenes	45	100	200
Staphylococcus aureus ATCC 6538	90	860	500
Enterococcus faecium DSM 2918	45	300	250
5°C			
Listeria monocytogenes	90	860	500
Staphylococcus aureus ATCC 6538	90	1100	750
Enterococcus faecium DSM 2918	90	450	500

Orth, R., and Mrozeck, H. 1989. Is the control of Listeria, Campylobacter, and Yersinia a disinfection problem? Fleischwirtsch, 69(10), 1575–1576.

Peracetic Acid Vapor

Compared to HP, peracetic acid vapor (PAAV) has some possible drawbacks. It is volatile (boiling point of 103°C, vapor pressure at 25°C 20 mm Hg); it has a sharp pungent odor; it has a flash point of 56°C, heated above which it is a possible fire and explosion hazard. It decomposes as the temperature is raised, and it is corrosive and toxic. Nevertheless its potent biocidal action captures our attention. Table 9–18 demonstrates the superiority of this vapor to other disinfectants in both speed of action and concentration required. Table 9–19 presents the data of Portner and Hoffman (1968), who found rapid reduction in number of spores in cold sterilization with PAAV. They found PAAV most effective at 80% relative humidity, lessening as the humidity was reduced, with very little activity at 20%. Spores on paper gave better results than spores on glass, apparently because of better penetration of the vapor into the paper. Doskocil and Fiser (1979) investigated PAAV for continual disinfection of air in rooms holding swine with

Table 9–11. *Bacteriostatic Activity of Disinfectants on* Streptococcus lactis *and* Streptococcus cremoris *in Cheese Vat (ppm for total inhibition of acid production)*

Disinfectant	ppm
Peracetic acid	5–35
Iodophor	75–200
Quaternary	>200
NaOCl	>400
Acid anionic	>600

Dunsmore, D.G., Makin, D., and Arkins, R. 1985. Effect of residues on five disinfectants in milk on acid production by strains of lactic starters used for cheddar cheesemaking and on organoleptic properties of the cheese. J. Dairy Res., 52, 287–297.

Table 9-12. *Inactivation of Viruses by Peracetic Acid (temperature = 20°C)*

Organisms	*Concentration (ppm)*	*Lethality (minutes)*	*Comments*	*Reference*
Poliovirus 1	400	5	7.5 $\log_{10}$ reduction	Kline and Hull, 1960
Cocksackievirus B-3	1280	5	5.5 $\log_{10}$ reduction	Kline and Hull, 1960
Cocksackievirus B-5	325	30	7.25 $\log_{10}$ reduction	Kline and Hull, 1960
Echovirus 10	1280	5	6.5 $\log_{10}$ reduction	Kline and Hull, 1960
Adenovirus 3,4,7	1280	5	4, 1.5, 3.5 $\log_{10}$ reductions	Kline and Hull, 1960
B virus	1280	5	7 $\log_{10}$ reduction	Kline and Hull, 1960
Herpes simplex	1280	5	3 $\log_{10}$ reduction	Kline and Hull, 1960
Enteric viruses	2000	10		Sprossig, 1975
Enteric viruses	2000	30		Harakeh, 1984
Human rotavirus	140	30		Harakeh, 1984
Simian rotavirus	20	30		Harakeh, 1984
Poliovirus 1	150–375,	60	DM	Baldry et al., in press
	> 750	30	DM	
	750–1500,	15	DM	
	1500–2250	10	DM	
Cocksackievirus	100–375	60	DM	Baldry et al., in press
	250–500	15	DM	Baldry et al., in press
Echovirus	100–375	60	DM	Baldry et al., in press
Phage MS2	12–15	5	DM	Baldry et al., in press
Phage ϕ, 174	25–30	5	DM	Baldry et al., in press
Poliovirus 1	375–750,	60	YE	Baldry et al., in press
	750–1500,	30	YE	Baldry et al., in press
	1500–2250,	15	YE	Baldry et al., in press
	> 2250	10	YE	Baldry et al., in press
Cocksackievirus	100–375,	60	YE	Baldry et al., in press
	500–1000	15	YE	Baldry et al., in press
Echovirus	100–375	60	YE	Baldry et al., in press
Phage MS2	75–94	5	YE	Baldry et al., in press
Phage ϕ, 174	94–113	5	YE	Baldry et al., in press

DM, demineralized water; YE, yeast extract

Table 9-13. *Sporicidal Activity of Peracetic Acid Against Spore-forming Bacteria and Bacterial Spores*

Organisms	*Concentration (ppm)*	*Lethality (minutes)*	*Temperature (°C)*	*Comments*	*Reference*
Bacillus stearothermophilus	100	15	20	BC	Gershenfeld and Davis, 1952
Bacillus stearothermophilus	2000	1	20	BC	Gershenfeld and Davis, 1952
Bacillus stearothermophilus	500	15	20	SS	Gershenfeld and Davis, 1952
Bacillus stearothermophilus	3000	1	20	SS	Gershenfeld and Davis, 1952
Bacillus coagulans 43-P	100	15	20	BC	Gershenfeld and Davis, 1952
Bacillus coagulans 43-P	2000	1	20	BC	Gershenfeld and Davis, 1952
Bacillus coagulans 43-P	500	10	20	SS	Gershenfeld and Davis, 1952
Bacillus coagulans 43-P	2000	1	20	SS	Gershenfeld and Davis, 1952
Bacillus subtilis	3000	10	20	BC, buffer	Greenspan and MacKellar, 1951
Bacillus subtilis	5000	10	20	BC, nutrient broth	Greenspan and MacKellar, 1951
Bacillus subtilis var. *niger* ATCC 9372	10,000	0.25	20	SS	Han et al., 1980
Bacillus stearothermophilus oxoid code BR 23	10,000	0.25	20	SS	Han et al., 1980
*Bacillus subtilis** ATCC 15441	25,000	1440	37	Carrier test	Baldry, 1983

BC, bacterial culture; SS, spore suspension
*Same results at pH 4, 7, and 9

Table 9–14. *Sporicidal Activity of Peracetic Acid: Effect of Temperature and Concentration*

Temperature (°C)	Minutes for Lethality at Concentration in ppm 5000	10,000	20,000	30,000	Organism	Reference
37	10	10	<0.5	<0.5	*Bacillus anthracis*	Hussaini and Ruby, 1976
20	20	10	5	<0.5	*B. anthracis*	Hussaini and Ruby, 1976
4	>60	20	20	<0.5	*B. anthracis*	Hussaini and Ruby, 1976
0	—	—	—	1	*B. subtilis* var. *niger*	Jones et al., 1967
−30	—	—	—	6	*B. subtilis* var. *niger*	Jones et al., 1967
−40	—	—	—	600	*B. subtilis* var. *niger*	Jones et al., 1967

Table 9–15. *Sporicidal Activity of Disinfectants to* Bacillus thermoacidurans *at 85°C—Effect of Solids*

Disinfectant	(ppm for Kill with %-Solids) 0	1
Peracetic acid	25	200
Chlorine-containing	50	400
Quaternaries	100	>400

Hutchings, I.J., and Xezones, H. 1949. Comparative evaluation of the bactericidal efficiency of peracetic acid, quaternaries and chlorine containing compounds. Proc. 49th Ann. Mfg. Soc. Am. Bacteriol. [Abstract], pp. 50–51.

atrophic rhinitis and obtained 91 to 94% kill of *Bordetella bronchiseptica* in 48 hours. Other workers showed that plastic tubing and medical instruments were sterilized in 2 hours at 22°C with PAAV.

Stability of Peracetic Acid

Peroxides in general are high-energy-state compounds, and as such can be considered thermodynamically unstable. PAA is considerably less stable than HP. 40% PAA loses 1 to 2% of its active ingredients per month, as compared with HP (30 to 90%), which loses less than 1% per year. The decomposition products of PAA are acetic acid, HP, oxygen, and water. Dilute PAA solutions are even more unstable: a 1% solution loses half its strength through hydrolysis in 6 days (Greenspan et al., 1955). PAA is produced by the reaction of acetic acid or acetic anhydride with HP in the presence of sulfuric acid, which acts as a catalyst, as shown:

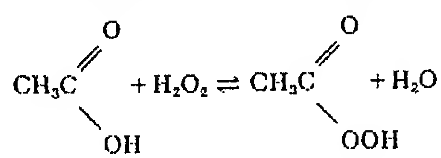

Table 9–16. *Sporicidal Activity of Disinfectants to* Bacillus anthracis *Spores with 4% Horse-Serum at 20°C*

Disinfectant	ppm	Time (Hours)
Peracetic acid	2500	0.5
Glutaraldehyde	20,000	2
Formaldehyde	40,000	2

Lensing, H.H. and Oei, H.L. 1984. Study of the efficiency of disinfectants against anthrax spores. Tijdschr. Diergeneeskd. *109*, (13), 557–563.

To prevent the reverse reaction, the PAA solution is fortified with acetic acid and HP. In addition a stabilizer is employed. This may be a sequestering agent (sodium pyrophosphate) or a chelating agent (8-hydroxyquinoline) that removes trace metals, which accelerate the decomposition of peroxides. (For a good discussion of stabilizers for peroxides, see Schumb et al., 1955.) A patented process employing anionic surfactants with dilute PAA solutions shows not only greater stability but greater antimicrobial activity (Bowing et al., 1977). Greenspan et al. (1955) demonstrated that although a 1% PAA solution at pH 2.5 lost 13.4% PAA in 1 day, at pH 7.0 it lost 84% in 1 day. On the other hand a formulation of 1% PAA, 14.5% acetic acid, 5.0% HP, 78.5% water, and 1% sulfuric acid lost only 2.7% in 84 days. To maintain stability, solutions must be made up with especially pure chemicals and deionized water, and kept free of dust and other contaminants. Commercial preparations with excellent stability are offered as shown in Table 9–20.

For stability PAA should be stored at ordinary, preferably cool temperatures in original containers. It is unaffected by glass and most plastics. It may extract the plasticizer from some vinyl formulations used as gaskets and will attack natural and synthetic rubbers (Dychdala, 1988). Pure aluminum, stainless steel, and tin-plated iron are resistant to PAA but plain steel, galvanized iron, copper, brass, and bronze are susceptible to reaction and corrosion (Schroeder, 1984).

Mechanism of Action of Peracetic Acid

Little work has been done to probe the mechanism of action of PAA as an antimicrobial agent. One can only speculate that it functions much as other peroxides and oxidizing agents. It is likely that sensitive sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites are oxidized and that double bonds are reacted. It is suggested that PAA disrupts the chemiosmotic function of the lipoprotein cytoplasmic membrane and transport through dislocation or rupture of cell walls (Baldry and Fraser, 1988). Its action as a protein denaturant may help to explain its action as a sporicide and ovicide.

Applications of Peracetic Acid

The powerful antimicrobial action of PAA at low temperatures along with the absence of toxic residuals has led to a wide range of applications. It has been accepted worldwide in the food processing and beverage industries, which include meat and poultry processing plants,

Table 9–17. *Bactericidal Activity of Disinfectants Against Test Bacteria in 10 Minutes by the Use Dilution Method—Based on 100% Active Ingredient*

Disinfectant	Composition	Bactericidal Concentration (ppm) against S. aureus	E. coli	P. vulgaris	P. aeruginosa
Persteril	40% peracetic acid	1,000	500	500	1,000
Chloramin	25% available chlorine	2,500	2,500	2,500	2,500
Wescodyne	1.6% available iodine	1,600	1,600	1,600	1,600
Jodoseptan	1.9% available iodine	1,520	950	1,520	1,900
Laurosept	25% laurylpyridinium bromide	2,500	2,500	2,500	25,000
Sterinol	10% dimethyl-lauryl-benzyl ammonium bromide	8,000	10,000	10,000	>10,000
Phenol	phenol	20,000	15,000	15,000	15,000
Lysol	50% cresol	40,000	40,000	40,000	40,000
Septyl	7.5% o-phenylphenol + 3.2% p-tert. amyl phenol	2,139	2,139	2,139	10,965
Formalin	37% formaldehyde	52,000	30,000	30,000	30,000
Alhydex	2% glutaraldehyde	6,000	5,000	5,000	5,000

Krzywicka, H., Jaszczuk, E., and Janowska, J. 1975. The range of antibacterial activity and the use concentrations of disinfectants. In *Resistance of Microorganisms to Disinfectants: Second International Symposium, Poznan*. Edited by W.B. Kedzia. Warsaw, Polish Academy of Sciences, pp. 89–91.

canneries, dairies, breweries, wineries, and soft drink plants (Dychdala, 1988), where it is said to be ideal for clean-in-place (CIP) systems. It is used as terminal disinfectant or sterilant for stainless-steel and glass tanks, piping, tank trucks, and railroad tankers (Interox Chemicals Ltd., undated). Its non-rinse feature, where its breakdown products in high dilution are not objectionable from the taste, odor, or toxicity standpoints, saves time and money. Schroder (1984) presented data on the breakdown time in water, beer, lemonade, and milk. Teuber (1978, 1979) studied the virucidal effectiveness on bacteriophages that are specific for *Streptococcus lactis* and *Streptococcus cremoris* and the sporicidal effectiveness against *Clostridium tyrobutyricum*. Binder and Foissy (1979) examined the fungicidal effect of the disinfectant containing peracetic acid with regard to its use as a room disinfectant in dairies. Jager and Puesopoek (1980) investigated PAA for the beverage industry and reported that all bacteria, yeasts, and fungi tested with 2500 ppm (0.25%) PAA were inactivated in 30 minutes, and with 5000 ppm in 15 minutes. A preparation of PAA (P-3-oxonia active) was approved by the FDA in 1986, giving clearance for its ingredients as indirect food additives in sanitizing solutions. Subsequently, EPA registration was issued and USDA authorization granted (Dychdala, 1988). In the food industry, plastic food con-

Table 9–18. *Resistance of Spores to Chemicals*

Chemical	Organism	Kill	Time (h)	Concentration (% w/v)	Temperature (°C)	Reference
Peracetic acid vapour	*B. subtilis* var. *niger*	10^5	0.02	0.0001	25	Portner and Hoffman (1968)
HCl vapour	*B. subtilis*	10^3	0.08	31*	20	Tuynenberg Muys et al. (1978)
Ethylene oxide	*B. subtilis*	10^4	0.7	0.07	40	Marletta and Stumbo (1970)
Hydrogen peroxide	*B. subtilis* var. *globigii*	10^3	0.17	25.8	24	Toledo et al. (1973)
Hypochlorous acid	*B. subtilis*	10^3	2.0	0.01†	10	Dye and Mead (1972)
Glutaraldehyde	*B. pumilis*	10^3	0.5	2.0	37	Thomas and Russell (1974)
Formaldehyde	*B. subtilis* var. *niger*	10^3	1.5	1.0	40	Trujillo and David (1972)
Propylene oxide	*B. subtilis* var. *niger*	10^3	17.0	0.1	37	Bruch and Koesterer (1961)
Sodium hydroxide	*B. subtilis*	10^3	24.5	5.0	40	Whitehouse and Clegg (1963)
Iodine (as an iodophor)	*B. subtilis*	10^2	>4.0	0.08	21	Cousins and Allan (1967)

*0.25 ml in a 300 ml bottle.

†Free chlorine.

Waites, W.M. 1982. Resistance of bacterial spores. In *Principles and Practice of Disinfection, Preservation, and Sterilization*. Edited by A.D. Russell and G.A.J. Ayliffe. Oxford, Blackwell Scientific Publications, p. 221.

Table 9–19. *The Effect of Peracetic Acid Vapor on* Bacillus subtilis *var.* niger *Spores at 80% Relative Humidity and 25°C; Spores on Paper and Glass; 1 mg/L Peracetic Acid*

	Paper		Glass	
Exposure (Minutes)	*Active spores*	*Sterile samples*	*Active spores*	*Sterile samples*
0	816,000	—	813,000	—
1.25	676	0	5	7
2.5	1	5	2	7
5	< 1	12	< 1	13
10	< 1	14	< 1	13
20	0	16	< 1	10

Portner, D.M., and Hoffman, R.K. 1968. Sporicidal effect of peracetic acid vapor. Appl. Microbiol., 16, 1782.

tainers were treated with a solution containing 0.1% PAA and 20% HP. They were sprayed on a conveyor belt using hot air to activate the solution and dry it off. Polyethylene strips with *Bacillus subtilis* spores were killed after treatment for 12 seconds at 65°C (Dallyn, 1980).

The properties of PAA have also been recognized by the medical community. The Centers for Disease Control of the United States have listed it as a chemical sterilant and high-level disinfectant (see Table 35–3). Application in the production of germ-free animals, where sterility is paramount, came very early (Greenspan et al., 1955). Wewalka and Werner (1973) found that 0.2% PAA killed *Mycobacterium tuberculosis* for the disinfection of respirators. Rubber tubes impregnated with *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* were disinfected with 0.5 or 1.0% PAA in 10 or 15 minutes, respectively. They recommended 0.5% PAA for 30 minutes for the disinfection of respirators, and stated that the large antimicrobial spectrum, short exposure time, and nontoxic decomposition products make peracetic acid a suitable disinfectant for medical machines. An automated machine to chemically sterilize medical, surgical, and dental instruments was developed using buffered PAA as the liquid sterilant (Steris System, 1988). It circulates the solution of PAA in a water bath at 50°C with an exposure time of 12 minutes. The tests reported showed spores of *Bacillus subtilis* and *Clostridium sporogenes* were all killed on 900 carriers in the AOAC carrier type test, and Steris System was granted FDA clearance to market the product. An interesting and novel application was described by Wutzler et al. (1975). They recommended 0.05 to 0.1% PAA to be used to sterilize serum and yeast-extract additions to mycoplasma-culture media, having found it to be as good as filtered components but less time- and equipment-intensive. Merka and Koubalik (1976) reported that a 5-minute immersion of rubber, steel, and plastic objects in 0.5% PAA provided sterilization, whereas 2% glutaraldehyde required 30 minutes immersion. Vashkov and co-workers (1974) found that 1% PAA took 20 to 45 minutes to sterilize medical instruments made of polymers and rubber, whereas 1% iodonate, 2% glutaraldehyde, or 6% hydrogen peroxide required more time.

In the preparation of pharmaceuticals, PAA permits the cold sterilization of emulsions, hydrogels, ointments, and powders. An oil-in-water emulsion contaminated with *Aspergillus* was sterilized in 10 minutes with 0.1% peracetic acid (Kuehn, 1978). Skin preparations containing zinc oxide and talc were preserved with 0.05 to 0.2%, yielding the same microbiocidal purity as hot-air–sterilized products (Kryzwicka et al., 1975). Zinc oxide lotions with 0.05 to 0.1% peracetic acid were tested on 26 patients for 1 year and did not cause skin irritation.

In industrial application, von Ballmoos and Soldavini (1959) reported on long-term experience with PAA in the disinfection of ion exchangers. They recommended the use of a 0.2% PAA solution for 1 hour to achieve complete disinfection. They found that the capacity of the respective cation and anion exchangers was not changed under the conditions used (0.2 to 1% PAA).

Microorganisms are now recognized as causing many problems in industrial water systems. These include microbially induced corrosion (see Chapter 53), plugging and fouling of heat exchangers, sprinklers, and cooling towers, and disease caused by the *Legionella* bacteria.

Table 9–20. *Commercial Peracetic Acid Equilibrium Solution—Approximate Chemical Composition (% by Weight)*

Ingredient	*FMC Corporation Philadelphia, PA 35% Peracetic Acid*	*FMC Corporation Philadelphia, PA 15% Peracetic Acid*	*Interox Chemicals England Proxitane 1507*	*Economics Lab. St. Paul, MN P3 Oxonia Active*	*Diversey Wyandotte Ontario S.T. 201 H*
Peracetic acid	35.5	15.0	15	5	14
H_2O_2	6.8	23.0	14	25	22
Acetic acid	39.3	16.0	28	6	28
H_2O	17.4	45.0	42	63	35
H_2SO_4	1.0	1.0	—	—	—
Stabilizer	0.05	0.05	< 1.0	1.0	1.0
	K=3.4	K=2.7	K=2.4	K=3.13	K=1.2

Baldry and Fraser (1988) reported on site trials for Legionella with 10 mg/L PAA on five cooling towers. In all cases the organism was eradicated in 20 minutes. Algae was controlled with 20 mg/L PAA twice a year, but a slug dose of 30 mg/L with 10 mg/L thereafter was better when there was heavy algae accumulation.

The effectiveness of PAA against bacteria and viruses led Baldry and French (1989) to investigate the use of PAA as a disinfectant for sewage and sewage effluents in laboratory and field trials. They found PAA to be an effective disinfectant for secondary effluent and stated that the ease of implementing PAA treatment without expensive equipment, the broad-spectrum activity even in the presence of organic matter, and the lack of environmentally undesirable byproducts make PAA appear favorable for sewage treatment processes. Baldry and Fraser (1988) reported on experiments with the disinfection of sewage sludge. They noted that 35 million wet tons of sewage sludge are distributed on grazing land each year in the United Kingdom. The sludge serves as a fertilizer but carries pathogens including Salmonella bacteria and ova of the beef tapeworm *(Taenia saginata)*. Disinfection of the sludge can eliminate it as a vehicle of transmission of infection to farm animals and also reduce the no-grazing interval of the land to which the sludge has been applied. Levels of 300 to 500 mg/L PAA reduced *Salmonella* levels below the limits of enumeration in all treated sludges, and even at 150 mg/L, the level has been reduced so that the sludge was considered safe for distribution to pasture land.

PAA functions against the cestode (tapeworm) oncospheres in sewage sludges producing lack of motion, dark coloration, granulation, and ovoid shrunken appearance (Fraser, 1986). In a digested sludge, 250 mg killed 99% of the embryos.

OTHER PEROXYGEN COMPOUNDS

In addition to PAA, other organic peroxy acids have been examined. Performic and perpropionic acids are similar in antibacterial activity to PAA, but performic is volatile and unstable, and perpropionic is more costly (Greenspan, 1946). Baldry and Fraser (1988) report that peroxyheptanoic and peroxynonanoic acids have higher activity on a molar basis than PAA. The perlauric acid has limited aqueous solubility but an antimicrobial spectrum somewhat like the quaternaries because of the long hydrocarbon chain. Monoperglutaric and diperglutaric acids and succinylperoxide are active, the latter having been marketed for disinfecting medical instruments. Derivatives of perbenzoic acid have been claimed in patents for sporicidal action. The magnesium salt of peroxyphthalate is a commercial product. It is a water-soluble solid effective against bacteria, yeasts, and spores (Baldry, 1984). Its activity is increased in acid solution, and with alcohol and with heat. It has been formulated as a sanitizer for bathrooms, kitchens, and diapers. As a powder with anionics it can be used for walls and floors in hospitals and is said to control hepatitis B and AIDS viruses. Eggensberger (1979) describes peracid powder disinfectants made in situ by adding water to mixtures of organic acid reservoirs (anhydrides, amides, and esters) to HP reservoirs such as sodium peroxide. Products based on this procedure are on the market in Germany for treating dentures and general hospital disinfection. Benzoyl peroxide is in general use in skin formulations for treating acne, and t-butyl hydroperoxide with phenols has been suggested for preventing microbial attack on engine fuels, cutting oils, and timber.

Inorganic peroxides have also been used to combat microbes. Perborates have been used in toothpastes and powders. Permanganate is antibacterial, antifungal, and antiviral; it was used as an antiseptic but its intense purple color is a disadvantage. It is said to be superior to copper sulfate as an algicide; 0.01% $KMnO_4$ kills algae in 4 to 6 hours in cooling towers (Fitzgerald, 1964, 1965). Calcium peroxide is reported to protect seed from microbial inhibition during sprouting. The alkali metal perdisulphates are strong oxidants but demonstrate little antimicrobial activity. However, monoperoxyysulfuric acid made from sulfuric acid and HP and partially neutralized with potassium hydroxide gives Caro's acid triple salt, which is used as a bleaching agent in toilet bowl and denture cleaners and as a swimming pool disinfectant. When investigated (Baldry, 1985), it showed activity against bacteria and viruses but none toward yeasts and fungi. However, with isopropanol it is synergistic and active toward yeast. With chloride or bromide it generates the hypohalite useful for slime control, pool disinfection, and sanitation of baby diapers (Gaya, 1979).

TOXICITY AND HAZARDS OF PEROXYGEN COMPOUNDS

Hydrogen peroxide is a clear, colorless liquid with a characteristic slightly acidic odor. It has low toxicity and is not a systemic poison because it is decomposed in the bowel before absorption (Gleason et al., 1969). Concentrated solutions are irritating to the skin, mucous membranes, and particularly to the eyes. The vapors can cause inflammation of the respiratory tract. It is not a carcinogen or a mutagen. Rubber gloves, safety goggles, and protective clothing should be worn when handling concentrated HP, PAA, or any liquid peroxygen compound or solution. They should be washed off immediately with large quantities of water if splashed on the skin or in the eyes. If swallowed, give milk or lukewarm water and call a physician (FMC Corp., undated).

PAA is a clear, colorless solution with a pungent odor, containing 40% or less of peracetic acid. The 40% solution has an LD_{50} to rats of 1540 mg per kilogram (National Institute of Safety and Health, 1974). Busch and Werner (1974) give LD_{50} values for rats of 315 for PAA and 263 for Wolfasteril, a preparation containing 36 to 40% PAA. For a 4% formulation (P-3 oxonia active) a value of 3.4 g per kilogram is given, which compares favorably to other common sanitizers (Dychdala, 1988). The subchronic oral feeding studies with this formulation showed